Detection of primary DNA damage: applicability to biomonitoring of genotoxic occupational exposure and in clinical therapy

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Abstract: The biological effect of putative genotoxic chemicals in the work place environment was monitored in peripheral mononuclear blood cells of exposed workers. DNA strand breaks, alkali-labile sites of DNA and DNA cross-links were measured using the alkaline filter elution method. A dose dependent increase in DNA damage was found in sterilization workers exposed to ethylene oxide and metal workers with exposure towards N-nitrosodiethanolamine. Two subpopulations with different response to the external exposure were found in nonsmoking sterilization workers. Nurses handling antineo-plastic agents without adequate safety provisions showed a statistically significantly higher rate of DNA strand breaks compared to other nurses handling cytostatic drugs with recommended safety equipment and also compared to not exposed controls. Also in several other occupational groups such as fire fighters possibly exposed to several genotoxic chemicals after an accident in a chemical plant, roofers and petrol pump attendants a significantly higher amount of DNA damage was found compared to controls. No statistically significant differences in the amount of DNA strand breaks were found in cabinet makers and car mechanics compared to controls. In peripheral mononuclear blood cells of ovarial carcinoma patients as well as of patients with Morbus Hodgkin an increased DNA strand break rate was found after application of cytostatic drugs. The individual patients showed a very different response after drug intake. The increase in DNA damage after drug application is possibly related to the success of the chemotherapy.

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Detection of primary DNA damage: applicability to biomonitoring of genotoxic occupational exposure and in clinical therapy

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The biological effect of putative genotoxic chemicals in the work place environment was monitored in peripheral mononuclear blood cells of exposed workers. DNA strand breaks, alkali-labile sites of DNA and DNA cross-links were measured using the alkaline filter elution method. A dose dependent increase in DNA damage was found in sterilization workers exposed to ethylene oxide and metal workers with exposure towards N-nitrosodiethanolamine. Two subpopulations with different response to the external exposure were found in nonsmoking sterilization workers. Nurses handling antineoplastic agents without adequate safety provisions showed a statistically significantly higher rate of DNA strand breaks compared to other nurses handling cytostatic drugs with recommended safety equipment and also compared to not exposed controls. Also in several other occupational groups such as fire fighters possibly exposed to several genotoxic chemicals after an accident in a chemical plant, roofers and petrol pump attendants a significantly higher amount of DNA damage was found compared to controls. No statistically significant differences in the amount of DNA strand breaks were found in cabinet makers and car mechanics compared to controls. In peripheral mononuclear blood cells of ovarial carcinoma patients as well as of patients with Morbus Hodgkin an increased DNA strand break rate was found after application of cytostatic drugs. The individual patients showed a very different response after drug intake. The increase in DNA damage after drug application is possibly related to the success of the chemotherapy.

Introduction

Monitoring the biological effect of putative genotoxic substances in human tissues *ex vivo* provides several advantages for the estimation of the occupational risk of humans compared to measuring the concentration of these chemicals in the environment or in body fluids of exposed persons. Individual differences in the uptake and excretion or in enzymatic activation and detoxication can be detected. Also modulating activity of chemicals in food or the leisure time environment should be observable. In many modern working places employees are exposed to a complex mixture of several chemicals. Moreover, not all genotoxic agents in the working place environment may be known prior to the investigation. The risk of the workers is then possibly underestimated when monitoring only selected chemicals. For risk estimation of workers due to their occupational environment we therefore recommend methods which are able to detect a biological effect rather than analysing selected chemicals.

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We used the detection of primary, including reversible damage of the DNA, namely DNA strand breaks, alkali-labile sites of DNA or DNA cross links, to monitor the biological effect of chemicals in humans. This type of DNA damage is produced by most genotoxic chemicals (Sina *et al.*, 1983). We have established the alkaline elution method for measuring DNA strand breaks in peripheral mononuclear blood cells obtained from humans exposed to putative carcinogens. Because the determination of genotoxic effects is directly done in freshly isolated human material no sophisticated extrapolation of results obtained from animals or *in vitro* systems is necessary.

Materials and methods

Alkaline elution

Twenty ml of venous heparinized blood were taken from the volunteers. All blood samples were kept on ice and processed within 4 h. Mononuclear blood cells were isolated by metrizoate-Ficoll centrifugation according to Boyum (1964).

The non-fractionated alkaline elution method was employed as described by Fuchs *et al.* (1994). In brief: a suspension of 1.5×10^6 mononuclear blood cells in 1 ml of ice-cold phosphate buffered saline was poured onto a polycarbonate filter with a pore size of 2 μm. Cells were lysed with 3 ml of a solution of 10 mM EDTA, 0.5% Triton X-100, 2 M NaCl (pH 10) and the DNA remaining on the filter was washed with 4.5 ml 10 mM EDTA (pH 10). The elution was performed at 4° C in the dark using a 5 M NaCl, 2 mM EDTA solution adjusted to pH 12.6 with NaOH for 10 h, resulting in a filtrate of single stranded DNA fragments of lower molecular weight. To remove the DNA of higher molecular weight retained on the filters these were sonicated in 15 ml elution buffer for 2×15 min. Quantification of the DNA was performed as described recently (Hengstler *et al.*, 1992). For standardization, DNA of untreated V79 cells was eluted simultaneously in every run on separate filters. V79 cells were cultured as described (Hengstler *et al.*, 1992). DNA concentrations of the eluted fractions and of the filter fractions were determined in triplicate. The elution rate was calculated as $-(\log_{10} R)/10$ h, where R represents the fraction of DNA remaining on the filter after 10 h of elution. For standardization the elution rate is expressed as the ratio of the elution rate of test cell DNA to the elution rate of V79 DNA. DNA of the peripheral mononuclear blood cells as well as DNA of the V79 cells was eluted on three separate filters each and mean values of these three elutions were calculated.

Description of subjects

Control persons were healthy volunteers, not on medication, without any known occupational exposure to chemicals or ionizing radiation and living in a moderately industrial town with about 200 000 inhabitants. Most of them were students or office employees. Workers were employed under regular working conditions mostly for several years in the same plant. Workers were in general healthy, with some complaining of moderately severe symptoms such as frequent sneezing, throat irritation, coughing, irritation of the eyes or skin eruption.

Statistical evaluations

The two-sided U-test according to Wilcoxon, Mann and Whitney was applied to evaluate statistical significance of differences between groups. The statistical significance of regression lines was evaluated using the t-statistics, with n being the number of individual measurements.

Results and discussion

In several occupational groups exposed to various chemicals under regular working conditions an elevated rate of DNA damage compared to the rate of DNA damage in apparently non-exposed controls could be detected.

In metal workers the genotoxic effect caused by the occupational exposure, measured as DNA single

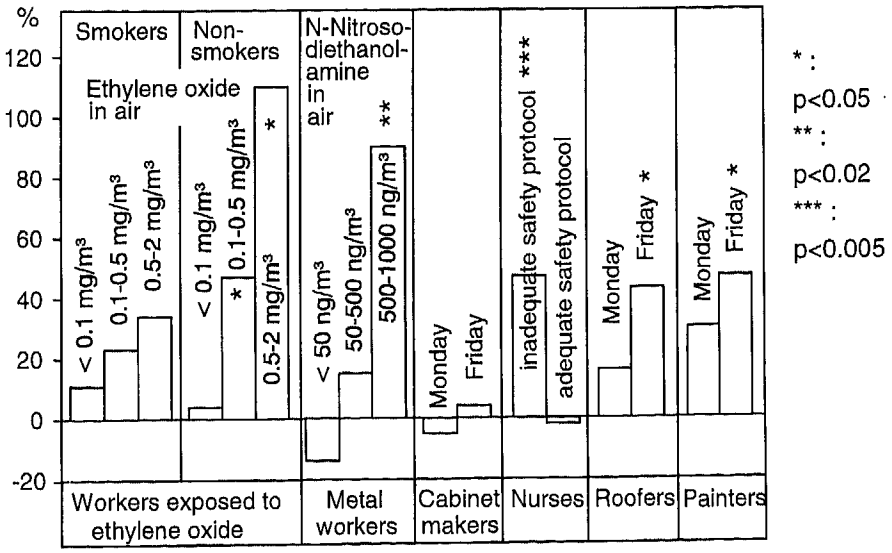

Fig. 1. Deviation of the mean elution rates of DNA of peripheral mononuclear blood cells from various groups of workers compared to that of controls without any apparent genotoxic exposure.

strand breaks in the mononuclear blood cells, was investigated. Metal workers using synthetic cutting fluids are potentially exposed to *N*-nitrosodiethanolamine (NDELA). NDELA is carcinogenic in experimental animals (Preussmann *et al.*, 1982; Lijinsky & Kovatch, 1985). DNA single strand breaks were detected in rat hepatocytes after incubation with NDELA *in vitro* as well as in rat liver cells after oral application of NDELA *in vivo* (Denkel *et al.*, 1986; Sterzel & Eisenbrand, 1986).

In this investigation NDELA was detected in the air of the working halls in a concentration up to 1000 ng m^{-3}. Workers in rooms with a mean concentration of *N*-nitrosodiethanolamine in the air of more than 500 ng m^{-3} revealed twice as many DNA strand breaks than workers in an environment with less than 50 ng m^{-3} (Fig. 1). Non-smoking workers with more than 4.5 h contact to cutting fluids per day showed a 1.5-fold higher mean elution rate of DNA than their non-smoking colleagues having had less than 4.5 h contact with cutting fluid ($p < 0.02$). For smoking metal workers only a small, statistically not significant difference in the mean elution rates of DNA of the two groups of workers was obtained. Also workers having had working place-related complaints showed a statistically significantly higher elution rate compared to workers without such complaints. No significant correlation was obtained between the extent of DNA damage and the estimated extent of skin contact or the concentration of NDELA found in the cutting fluids. Therefore, for workers in this investigation NDELA incorporated by inhalation is probably more relevant for genotoxic damage than NDELA resorbed by skin.

In sterilization workers exposed to ethylene oxide we could demonstrate the consequence of a polymorphism in the human population on the genotoxic potency of ethylene oxide. Ethylene oxide is carcinogenic in experimental animals. In rats mononuclear cell leukemia, peritoneal mesotheliomas and primary brain tumours were found (Lynch *et al.*, 1984; Snellings *et al.*, 1984). Evidence for an increased incidence of leukemia and stomach cancers in humans occupationally exposed to ethylene oxide but possibly also to other chemicals was found (Hogstedt *et al.*, 1979, 1986). In the present investigation a dose dependent increase in DNA strand breaks in peripheral mononuclear blood cells of workers exposed to ethylene oxide was detected (Fuchs *et al.*, 1994). The non-smoking workers revealed inhomogeneous response in generating DNA strand breaks with respect to the external pollution. While some of the workers showed only a minor induction of DNA strand breaks, for other workers employed in rooms with the same concentrations of ethylene oxide in the air of the working areas a much stronger increase in the rate of DNA strand breaks was observed. According to this observation the workers were classified into two subpopulations. Each of the two subpopulations showed a highly significant linear correlation between the DNA strand breaks and the ethylene oxide concentration. The majority of the non-smoking workers (67%) were strongly sensitive to ethylene oxide. For the 'highly sensitive' group a slope of 1.1 m^3 mg^{-1} and for 'lower sensitive' group an approximately five-fold lower slope (0.19 m^3 mg^{-1}) was calculated. None of the other parameters measured or investigated, such as age, sex, details of occupational operation, alcohol consumption or the excess of skin contact to ethylene oxide, was significantly different in the two subpopulations. The smoking workers, however, could not be divided into subpopulations solely based on the response of the individuals to the extent of external exposure. Two groups with a different degree of DNA damage were also found after incubation of venous blood of non-smokers with ethylene oxide *in vitro* but not after *in vitro* incubation of isolated peripheral mononuclear blood cells.

Our current working hypothesis is that a polymorphism in detoxifying enzymes in blood tissues accounts for the different susceptibility of humans to ethylene oxide. Ethylene oxide is detoxified either by conjugation to glutathione or by enzymatic hydrolysis. For the glutathione transferase GSTM1 a polymorphism in human lymphocytes and in human liver is known (Seidegard *et al.*, 1985). Recently Hallier *et al.* (1993) found a polymorphism in glutathione transferase activity for small molecules such as methyl bromide or dichloromethane and possibly ethylene oxide in human erythrocytes. The enzyme was characterized as a glutathion *S*-transferase class θ (GSTT1) by Pemble *et al.* (1994). A genetic polymorphism was found in humans with 62% of the population possessing the intact gene. We have genotyped 45 individuals with respect to GSTM1 and GSTT1 by PCR. Our results showed that the polymorphism in the extent of DNA damage in peripheral mononuclear cells found after incubation of blood with ethylene oxide *in vitro* cannot satisfactorily be explained by a polymorphism in either GSTM1 or GSTT1.

In a group of nurses preparing cytostatic drugs without adequate safety precautions a significantly higher mean elution rate (47%) was observed compared to controls ($p < 0.01$), After installation of laminar flow safety hoods and the recommendation of a safety protocol the DNA strand break rates for the nurses declined to the level of the controls. In several other groups of nurses preparing cytostatic drugs all of whom applied safety precautions such as the usage

of laminar flow safety hoods, gloves or surgical masks no statistically significantly elevated strand break rates compared to controls were observed.

DNA single strand breaks were detected in mononuclear blood cells of fire fighters exposed to *o*-nitroanisole and other substances released during an accident in a chemical plant. The extent of DNA single strand breaks in the exposed fire fighters was compared to that of a matched group of fire fighters not occupied in the contaminated region and to that of non-exposed controls. The number of DNA single strand breaks of the exposed fire fighters 19 days after the exposure was weakly but significantly increased when compared to the data either obtained from non-exposed fire fighters (22% increase, $p < 0.05$) or from persons without any apparent genotoxic occupational exposure (27% increase, $p < 0.05$). In a second analysis, performed 3 months after the first one, the number of DNA single strand breaks in the exposed fire fighters was no longer increased compared to the control groups.

In several other occupational groups such as roofers, non-smoking petrol pump attendants with a daily service time of more than 4 h or painters (Fig. 1) an increased rate of DNA strand breaks was detected compared to controls. No significant differences were found in cabinet makers or car mechanics compared to controls.

DNA strand breaks and DNA cross-links were detected in mononuclear blood cells of 15 ovarian carcinoma patients (Hengstler *et al.*, 1992). These patients received therapy with cyclophosphamide and carboplatin. The patients showed an increased elution rate of 37% compared to healthy controls prior to the current cycle of chemotherapy, probably due to treatment in a previous cycle of therapy. At the end of the actual cycle an average acceleration of the elution rate of 157% was found compared to controls. The amount of DNA-protein cross-links was also increased after drug application.

The individual patients showed different responses after drug intake. While some patients showed hardly any alteration in the elution rate, others showed an acceleration of up to 400%. Monitoring the course of disease in six of these patients indicated that a strong acceleration in the elution rate after drug application was linked to the success of the chosen cancer treatment as measured by a decrease in the tumour marker CA12-5 to the normal level.

In another study patients with Morbus Hodgkin (stage II–IV according to the Ann-Arbor-Classification) treated with COPP/ABVD-chemotherapy were examined. COPP/ABVD-chemotherapy includes application of cyclophosphamide (day 1, 8), vincristine (day 1, 8), procarbazine (day 1–14), prednisone (day 1–14), doxorubicin (day 29, 43), bleomycin (day 29, 43), vinblastine (day 29, 43) and dacarbazine (day 29, 43). Usually three cycles of therapy are given. DNA single strand breaks were detected in mononuclear blood cells of the patients before and 16–18 h after the first cyclophosphamide application (day 1). Mean values of the increase in elution rates of the first and second cycle of therapy were calculated. The success of the therapy was classified into complete response (all tumours disappeared for at least 4 weeks), partial remission (more than 50% decrease in the size of tumours for at least 4 weeks), no change (less than 50% decrease in the size of tumours) and progression (the size of the tumours increased). Also in this investigation, a correlation between the success of the therapy and the increase in elution rates could be observed. However, a higher number of cases are needed for statistical evaluation. In the four patients with a complete response after therapy a mean increase in elution rate of 1.85 ± 0.32 (mean $\pm$ SE) after the first application was observed. Four patients with partial response showed a mean increase in elution rate of 1.10 ± 0.19 and for the one patient with progression of the tumour the lowest increase in elution rate of 0.52 was obtained.

In summary we could demonstrate that using the alkaline elution method to measure DNA damage in peripheral mononuclear blood cells is a powerful tool to evaluate the genotoxic risk of workers. In sterilization workers exposed to ethylene oxide or in metal workers exposed mainly to *N*-nitrosodiethanolamine a dose dependent increase in DNA single strand breaks was observed. The applicability of the method for a short-term control of the success of safety precautions against putative carcinogens was demonstrated by investigating nurses preparing cytostatic drugs prior to and after installation of safety equipment. The method was also applied to estimate the genotoxic risk of fire fighters who were exposed to genotoxic substances after an accident in a chemical plant. Although established only with a limited number of patients, it already appears convincingly clear that we can use this method to predict the success of some anticancer chemotherapies at an early stage of treatment. The alkaline elution method can also be applied if the genotoxic chemicals in the environment are unknown. Contrary to epidemiological carcinogenicity studies, results of the alkaline elution can be obtained soon after exposure. The detection of DNA strand breaks can, therefore, be applied as an early warning indicator in the case of a genotoxic environmental pollution.

References

Boyum A. Separation of white blood cells. *Nature (London)* 1964: **204**, 793.

Denkel E, Pool BL, Schlehofer JR, Eisenbrand G. Biological activity of *N*-nitrosodiethanolamine and of potential metabolites which arise after activation by alcohol dehydrogenase in Salmonella typhimurium, in mammalian cells and *in vivo*. *J Cancer Res Clin Oncol* 1986; **111**, 149–153.

Fuchs J, Wullenweber U, Hengstler JG, Bienfait HG, Hiltl G, Oesch F. Genotoxic risk for humans due to work place exposure to ethylene oxide: remarkable individual differences in susceptibility. *Arch Toxicol* 1994: **68**, 343–348.

Hallier E, Langhof T, Dannappel D, Leutbecher M, Schröder K, Goergens HW, Müller A, Bolt HM. Polymorphism of glutathione conjugation of methyl bromide, ethylene oxide and dichloromethane in human blood: influence on the induction of sister chromatid exchanges (SCE) in lymphocytes. *Arch Toxicol* 1993: **67**, 173–178.

Hengstler JG, Fuchs J, Oesch F. DNA strand breaks and DNA cross-links in peripheral mononuclear blood cells of ovarian cancer patients during chemotherapy with Cyclophosphamide/Carboplatin. *Cancer Res* 1992: **52**, 5622–5626.

Hogstedt C, Rohlen O, Berndtson BS, Axelson O, Ehrenberg L. A cohort study of mortality and cancer incidence in ethylene oxide production workers. *Br J Ind Med* 1979: **36**, 276–280.

Hogstedt C, Aringer L, Gustavsson A. Epidemiologic support for ethylene oxide as a cancer-causing agent. *JAMA* 1986: **255**, 1575–1578.

Lijinsky W, Kovatch RM. Induction of liver tumors in rat by *N*-nitrosodiethanolamine in low doses. *Carcinogenesis* 1985: **6**, 1679–1681.

Lynch DW, Lewis TR, Moorman WJ, Burg JR, Groth DH, Khan A, Ackerman LJ, Cockrell BY. Carcinogenic and toxicologic effects of inhaled ethylene oxide and propylene oxide in F344 rats. *Toxicol Appl Pharmacol* 1984: **76**, 69–84.

Oesch F, Hengstler JG, Fuchs J. Cigarette smoking protects mononuclear blood cells of carcinogen exposed workers from additional work-exposure-induced DNA single strand breaks. *Mutat Res* 1994: **321**, 175–185.

Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, Ketterer B, Taylor JB. Human glutathione *S*-transferase Theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. *Biochem J* 1994: **300**, 271–276.

Preussmann R, Habs M, Habs H, Schmähl D. Carcinogenicity of *N*-nitrosodiethanolamine in rats in five different dose levels. *Cancer Res* 1982: **42**, 5167–5171.

Seidegard J, Pero RW. The hereditary transmission of high glutathione transferase activity towards *trans*-stilbene oxide in human mononuclear leucocytes. *Hum Genet* 1985: **69**, 66–68.

Sina JF, Bean CL, Dysart GR, Taylor VI, Bradley MO. Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. *Mutat Res* 1983: **113**, 357–391.

Snellings WM, Weil CS, Maronpot RR. A two-year inhalation study of the carcinogenic potential of ethylene oxide in Fischer 344 rats. *Toxicol Appl Pharmacol* 1984: **75**, 105–117.

Sterzel W, Eisenbrand G. *N*-Nitrosodiethanolamine is activated in the rat to an ultimate genotoxic metabolite by sulfotransferase. *J Cancer Res Clin Oncol* 1986: **111**, 20–24.